

Docket: NEB-177-PUS



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Evans et al.

EXAMINER: Schnizer

SERIAL NO.: 09/937,070

GROUP: 1656

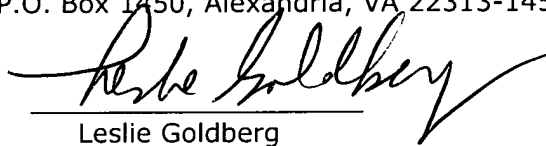
FILED: January 29, 2002

FOR: Method for Producing Circular or Multimeric Protein Species
in vivo or *in vitro* and Related Methods

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Leslie Goldberg

Sir:

DECLARATION UNDER 37 C.F.R. §1.131


As a below named inventor, I hereby declare that:

1. My name is Dr. Ming Xu, Senior Scientist at New England Biolabs Inc. My resume is attached.
2. The Examiner has rejected claims 12, 14 and 16 in the above application, directed to a method for the *in vivo* production of a cyclic

polypeptide, as invented first by Scott et al. PNAS 96, pp 13638-13643 published November 23, 1999.

3. However, the present claimed invention was invented prior to November, 1999 as recorded in dated pages in my laboratory notebook.

4. I further declare under penalty of perjury pursuant to laws of the United States of America, the foregoing is true and correct.



Dr. Ming Xu

Jan. 27th, 2006
Date

Ming-Qun Xu, Ph.D.

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Education:

B.S. 1982 University of Science and Technology of China

Ph.D. 1989 Molecular Biology, Department of Biological Sciences, State University of New York at Albany

Work History

1990-1992: Postdoctoral research on self-splicing introns with Dr. David Shub, SUNY at Albany

Discovered the first eubacterial intron (Xu et al. Science, 1990)

1992 – 1994: Postdoctoral research with Dr. Fran Perler at New England Biolabs, Inc. Performed the first in vitro protein splicing experiment (Xu et al., Cell 1993)

1994 – 1997: Staff Scientist at New England Biolabs, Inc. Investigated the chemical mechanism of protein self-splicing. Developed the intein-based affinity purification system – IMPACT.

1997 – present: Senior Scientist at New England Biolabs, Inc. Structural and mechanistic studies of self-splicing inteins. Engineered inteins for protein semisynthesis, protein backbone cyclization and trans-splicing.

2001-2005: Managing Director, New England Biolabs (Beijing) Ltd.

Current Research Interest:

Structural and mechanistic studies of protein splicing have been conducted by collaboration to solve the crystal structures of self-splicing-inteins derived from the *dnaB* and *dnaE* genes of *Synechocystis* sp. PCC6803. The finding that the DnaE intein precursor structure contains a zinc ion, the only identified inhibitor of both *cis*- and *trans*-splicing, chelating the highly conserved Cys160 and Asp140 reveals the structural basis of Zn²⁺-mediated inhibition. These structural

studies provide insight into the sequential reaction property of protein splicing as well as the strategies to utilize inteins for protein engineering.

A number of intein engineering projects have been carried out for protein/antibody affinity purification, protein labeling and tagging, ligation and cyclization of expressed proteins. The Intein-mediated protein ligation (IPL) method has been applied to new fields including antibody characterization, epitope mapping, kinase/phosphatase assays for analysis via peptide arrays, western blots and ELISA.

PUBLICATIONS:

1. Ming-Qun Xu, Inca Ghosh, Samvel Kochinyan and Luo Sun. Intein-mediated Peptide Arrays for Epitope Mapping and Kinase/Phosphatase Assays. *Methods in Molecular Biology*, vol., *Microarrays: Methods and Protocols* Edited by J.B. Rampal. Humana Press Inc., Totowa, NY. In press.
2. Sun Ping, Sheng Ye, Sebastien Ferrandon, Evans, T.C. Jr., Ming-Qun Xu, Zihao Rao (2005) Crystal structures of an intein from the split *dnaE* gene of *Synechocystis* sp. PCC6803 reveal the catalytic model of intein without the penultimate histidine and the mechanism of zinc ion inhibition of protein splicing. *J. Mol. Biol.* 353: 1093-1105.
3. Xu, M.-Q. and Evans, T.C. Jr. (2005) Recent Advances in Protein Splicing: Manipulating Proteins In Vitro and In Vivo. *Curr. Opin. Biotechnol.* 16, 440-446.
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5. Shaorong Chong and Ming-Qun Xu. (2005) Harnessing Inteins for Protein Purification and Characterization. Marlene Belfort (Ed.) *Nucleic Acids and Molecular Biology*, Vol. 16, 273-292.
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9. Xu, J., Sun, L., Ghosh, I., and Xu, M.-Q. (2004) Western blot analysis of Src kinase assays using peptide substrates ligated to a carrier protein. *Biotechniques*. 36:976 -981.
10. Li ZY, Li YJ, Guo CY, Shi YW, Xu MQ, Trommer WE, Yuan JM. (2004) Soluble expression and affinity purification of functional domain of human acetylcholine receptor alpha-subunit by the modulation of maltose binding protein. *Biotechnol Lett*. 2004 Dec;26(23):1765-9.

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13. Yi Ding, Ming-Qun Xu, Inca Ghosh, Xuehui Chen, Sebastien Ferrandon, Guillaume Lesage, Zihao Rao (2003) Crystal structure of a mini-intein reveals a conserved catalytic module involved in side chain cyclization of asparagine during protein splicing. *J. Biol. Chem.* 278, 39133-39142.
14. Hang Gyeong Chin et al. (2003) Protein trans-splicing in transgenic plant chloroplast: Reconstitution of herbicide resistance from split genes. *PNAS* 100, 4510-4515.
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16. Evans, T.C. Jr. and M.-Q. Xu. (2002) Purification of recombinant proteins from *E. coli* by engineered inteins. *Methods in Molecular Biology, vol. 205, E. coli Gene Expression Protocol Edited by P.E. Vaillancourt. Humana Press Inc., Totowa, NY*
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23. Poland, B. W., Xu, M.Q., Quijcho, F.L (2000). Structural Insights into the Protein Splicing Mechanism of PI-SceI. *J. Biol. Chem.* 275, 16408-16413.
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